PAN-PACIFIC ENTOMOLOGIST
68(2): 100–104, (1992)

SPHERICAL HYPHAL BODIES OF *PANDORA NEOAPHIDIS* (REMAUDIÈRE & HENNEBERT) HUMBER (ZYGOMYCETES: ENTOMOPHTHORALES) ON *ACYRTHOSIPHON PISUM* (HARRIS) (HOMOPTERA: APHIDIDAE): A POTENTIAL OVERWINTERING FORM

MING-GUANG FENG,[1] ROBERT M. NOWIERSKI,[1] ROBERT E. KLEIN,[2] ALBERT L. SCHAREN,[3] AND DAVID C. SANDS[3]

[1]Entomology Research Laboratory, Montana State University, Bozeman, Montana 59717

[2]Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, Washington 99350

[3]Department of Plant Pathology, Montana State University, Bozeman, Montana 59717

Abstract.—Cadavers of the pea aphid, *Acyrthosiphon pisum* (Harris), occurred abundantly on commercial alfalfa in Kennewick, Washington, during late autumn of 1990. An aphid-specific fungal pathogen, *Pandora neoaphidis* (Remaudière & Hennebert) Humber, was responsible for the death. Numerous hyphal bodies of the fungus inside the cadavers were spherical and averaged 11.5 (9.3–15.0) μm in diameter (n = 100). Such spherical hyphal bodies apparently developed from regular hyphal bodies forming septa, which has never been recorded for *P. neoaphidis*. Over 300 cadavers collected in the field on 15 Oct were randomly sorted into three batches and then maintained under different environmental conditions for studying the overwintering potential of the fungus. Cadavers maintained in a dark refrigerator at approximately 4° C or placed within nylon-chiffon mesh bags (ca. 5 × 5 mm) and secured to the branches of shrubs (approximately 0.5 m above the ground in Bozeman, Montana) were capable of producing conidia and infecting aphids in monthly observations from November to April with consistently visible snow cover. In contrast, cadavers placed in polypropylene microcentrifuge tubes (38 × 13 mm), corked with sterile cotton and then buried in the field soil (approximately 6 cm deep), were found to have exhausted all their sporulation potential and infective capability in the first observation of late November. The results indicate that *P. neoaphidis* may survive winter months in the form of hyphal bodies on plant substrates above the ground rather than in the soil.

Key Words.—Insecta, *Acyrthosiphon pisum,* Entomophthorales, *Pandora neoaphidis,* aphid-specific fungal pathogen, overwintering

---

A mycosis of the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), was observed in a commercial alfalfa field in Kennewick, Washington, during late September through mid-October, 1990. Alfalfa stems were heavily infested with aphids (100% of the plants infested and more than 100 aphids per alfalfa stem). Aphid cadavers, resulting from fungal infection, were observed in abundance. Approximately 10% of the axillary shoots on alfalfa stems contained at least one cadaver, and some of the shoots contained 10 or more.

Aphid cadavers collected on 27 Sep, 1 Oct and 9 Oct (the last date coinciding with alfalfa harvest) were shipped via overnight mail to MGF in Bozeman, Montana, for identification of pathogens involved. The aphid-specific fungus, *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Zygomycetes, Entomophthora-

les), was found to be the only pathogen responsible for the mycosis observed. This was based on microscopic examination of nearly 200 cadavers individually mounted on slides with aceto-orcein following maintenance in a moist chamber at approximately 25° C for 20 h. No secondary infection by other entomophthoralean fungi was detected.

Morphological features including conidiophores, conidia (Fig. 1a) and hyphal bodies (Fig. 1b) coincided well with those previously documented for *P. neoaphidis* (e.g., Feng et al. 1990). Measurements of 100 primary conidia randomly taken from 20 slides (cadavers) averaged 22.0 (17.5–27.5) × 11.3 (9.25–14.3) μm, falling within the previously defined range of *P. neoaphidis* (Waterhouse & Brady 1982).

Spherical hyphal bodies (SHB) (Figs. 1c, 1d, 1h), not previously documented for *P. neoaphidis,* appeared with primary conidia and regular hyphal bodies (RHB) in all the cadavers examined. The relative abundance of these unusual hyphal bodies seemed to be negatively correlated with the abundance of primary conidia and RHBs. Some of the cadavers were nearly filled with SHBs. The frequency of cadavers containing SHBs tended to increase with each successive collection date.

The SHBs measured 11.5 (9.3–15.0) μm in diameter ($n = 100$), and were nearly equal to the diameter (width) of primary conidia (Fig. 1a) and RHBs (Fig. 1b). Like uninucleate primary conidia of *P. neoaphidis,* most SHBs contained only a single large nucleus (Fig. 1c). Some SHBs were found to have two or more nuclei (Fig. 1d). SHBs with multiple nuclei were usually larger in size than those with only one nucleus.

The SHBs apparently developed from RHBs, as shown in Figs. 1e–1h. A septum sometimes appeared in the hyphal body, preceding the formation of a SHB (Figs. 1e–1g). Septa are usually absent from the vegetative cells in the Entomophthoraceae (Humber 1989) and have never been recorded for *P. neoaphidis.* Subsequently, the single cell separated by a septum became spherical, often at the end of the hyphal body (Fig. 1g). Eventually, the remainder of the hyphal body gradually disappeared as its contents (protoplasts) entered the new SHBs (Fig. 1h).

The appearance of SHBs late in the season suggests that SHBs may function as an overwintering form in the life cycle of *P. neoaphidis.* This hypothesis was tested by tracing the infectivity of cadavers collected from the field, then exposed to different environments during winter months. Over 300 cadavers were collected from uncut alfalfa plants on the border strips of the field in Kennewick on 15 Oct and carried back to the laboratory in Bozeman. These cadavers were then separated into 3 batches and about 15 from each batch were placed into polypropylene microcentrifuge tubes (38 × 13 mm) corked with sterile cotton for two batches or nylon-chiffon mesh bags (approximately 5 × 5 cm; four threads per mm) for the third batch. The two batches of tubes were maintained in a dark refrigerator of about 4° C and buried in the field soil (approximately 6 cm deep) on the Montana State University campus, respectively. Mesh bags of the third batch were secured to the branches of outdoor shrubs approximately 0.5 m above the ground on the university campus. Thereafter, one tube or bag of cadavers was randomly taken from each batch every month and used to inoculate aphids reared in the laboratory by suspending the cadavers over the aphids for a spore shower. It was found that cadavers hung in bushes or maintained in the refrigerator were capable of producing conidia and infecting aphids throughout the cold winter with consistently visible snow cover from November to April. However, cadavers in the tubes

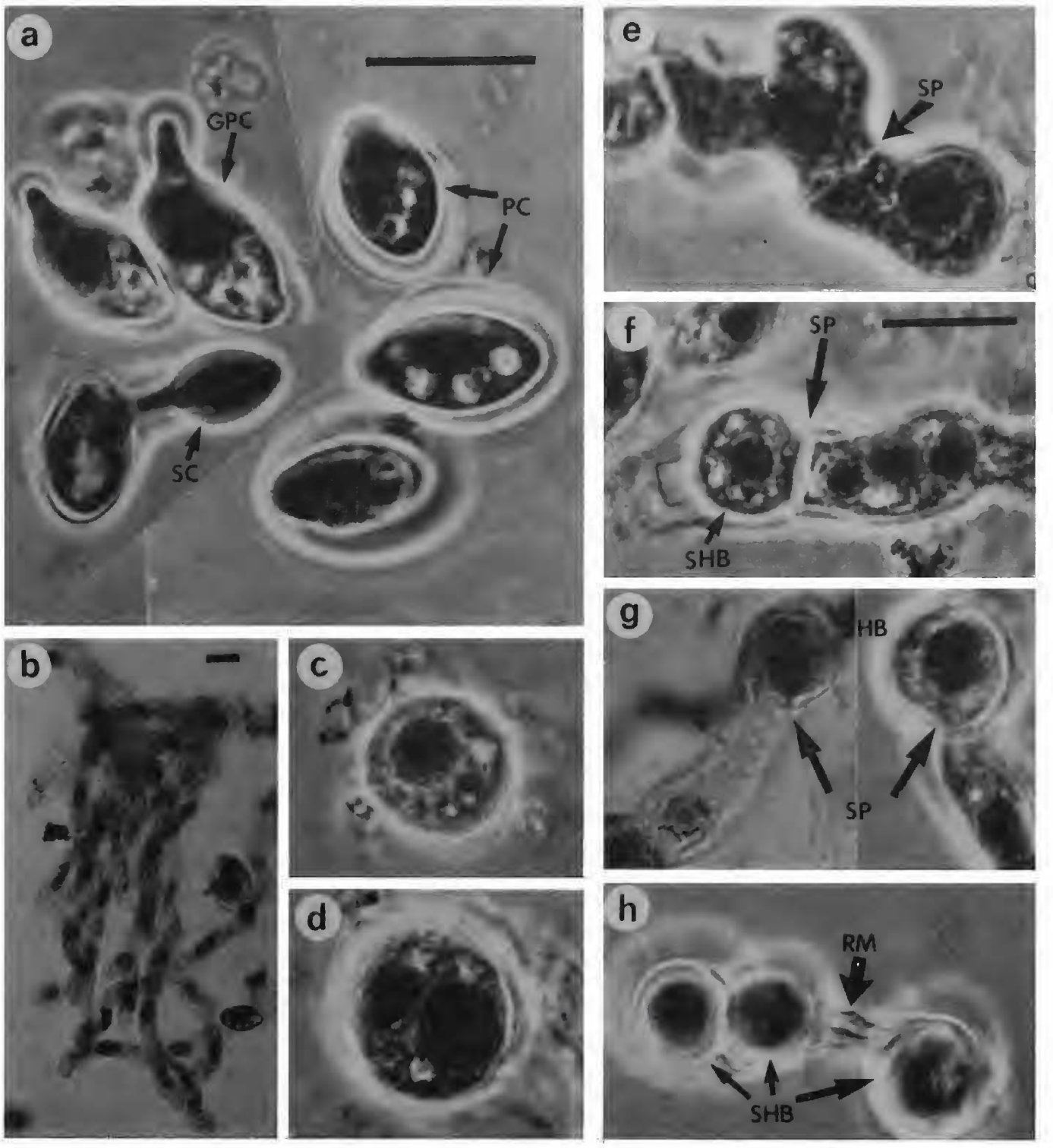

Figure 1. Common morphological characteristics (a, b) and unusual spherical hyphal bodies [SHB] (c–h) of *P. neoaphidis* associated with cadavers of *A. pisum* on alfalfa in Kennewick, Washington, in autumn. (a) Primary conidia [PC], germinating PC [GPC] and secondary conidium [SC] formed from PC. (b) Regular hyphal bodies [RHB]. (c) SHB uninucleate. (d) SHB binucleate. (e, f) Well-defined septum [SP], at arrow, seen in RHB. (g) SHB forming at the end of RHB. (h) Newly-formed SHB and remains [RM] of RHB. Scale bars: 20 μm; the bar for (a) also applies to (c–e) and (g, h).

buried in the soil were found to have exhausted all their sporulation potential in the first observation on 30 Nov. A layer of conidia were then visible on the inside wall of the tube and the cadavers became indistinguishable from one to another. As a result, the cadavers in the soil could not infect aphids at that time. This appeared to be attributable to the high humidity in the soil under snow cover during the relatively mild November.

Therefore, our observations indicate that the *P. neoaphidis* hyphal bodies in aphid cadavers can survive winter months only in relatively dry environments

(e.g., on plant substrates above the ground) rather than in the moist soil, as postulated by some authors (e.g., Latteur & Godefroid 1983). This is similar to a report that hyphal bodies of *P. neoaphidis* may maintain infectivity in cadavers for up to 32 weeks at regimes of 0° C and ≤50% relative humidity (Wilding 1973). In contrast, other entomophthoralean fungi generally overwinter as resting spores, as seen in *Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller (Latgé et al. 1978) and *Zoophthora radicans* (Brefeld) Batko (Perry & Régnière 1986). Although it was claimed that resting spores of *P. neoaphidis* had been obtained in vitro (Uziel & Kenneth 1986), resting spores have never been observed from aphids infected by *P. neoaphidis* in the field. The SHBs observed in this study are unlikely to be resting spores (zygospores or azygospores) because they are thin-walled and too small for resting spores typically reported for the Entomophthorales (R. A. Humber, personal communication). Resting spores have been observed in the field for another entomophthoralean species, *Entomophthora planchoniana* Cornu, but the primary overwintering form of this latter fungus is hyphal bodies that are distinct from those usually found for the same species (Keller 1987). SHBs in the pea aphids infected by *P. neoaphidis* late in the season seem to be analogous to the hyphal bodies of *E. planchoniana.*

It remains unknown what environmental stimuli may induce the information of septa in the hyphal bodies, thus forming the SHBs. During the period from 16 Sep to 15 Oct, 1990, local day length decreased from about 12.5 h to 11 h, while the daily minimum temperature was 9.3 (range: 1.1–16.1)° C, daily maximum 22.4 (10.6–30.6)° C, and daily mean 15.7 (7.8–22.8)° C. Whether these environmental conditions (short day and low temperature) may be conducive to physiological changes in the aphid hosts, which in turn may influence fungal development, is unclear.

Finally, *P. neoaphidis* may require a variety of host species from different crops or non-crop plants to complete the life cycle. Plant hosts that remain in the field through late autumn or are perennial (e.g., alfalfa) may provide a source of inoculum to initiate infections in aphid populations that infest spring and summer crops the following year (e.g., small grains). This hypothesis warrants further studies.

Acknowledgment

We thank R. A. Humber, S. A. Woods and D. A. Streett for their critical review of this manuscript. This article is published as Journal Series No. J-2587 from the Agricultural Experiment Station, Montana State University, Bozeman, Montana.

Literature Cited

Feng, M. G., J. B. Johnson & L. P. Kish. 1990. Survey of entomopathogenic fungi naturally infecting cereal aphids (Homoptera: Aphididae) of irrigated grain crops in southwestern Idaho. Environ. Entomol., 19: 1534–1542.

Humber, R. A. 1989. Synopsis of a revised classification for the Entomophthorales (Zygomycotina). Mycotaxon, 34: 441–460.

Keller, S. 1987. Observations on the overwintering of *Entomophthora planchoniana.* J. Invertebr. Pathol., 50: 333–335.

Latgé, J. P., D. Perry, B. Papierok, J. Coremans-Pelseneer, G. Remaudière & O. Reisinger. 1978.

Germination d'azygospore d'*Entomophthora obscura* Hall & Dunn, role du sol. C.R. Acad. Sci. Paris Ser. D, 287: 946–946.

Latteur, G. & J. Godefroid. 1983. Trial of field treatments against cereal aphids with mycelium of *Erynia neoaphidis* (Entomophthorales) produced in vitro. pp. 2–10. *In* Cavalloro, R. (ed.). Aphid antagonists. A.A. Balkema, Rotterdam.

Perry, D. F. & J. Régnière. 1986. The role of fungal pathogens in spruce budworm population dynamics: frequency and temporal relationships. pp. 167–170. *In* Samson, R. A., J. M. Vlak & D. Peters (eds.). Fundamental and applied aspects of invertebrate pathology. Wageningen, Netherlands.

Uziel, A. & R. G. Kenneth. 1986. In vitro resting-spore formation in *Erynia neoaphidis*. p. 230. *In* Samson, R. A., J. M. Vlak & D. Peters (eds.).Fundamental and applied aspects of invertebrate pathology. Wageningen, Netherlands.

Waterhouse, M. & B. L. Brady. 1982. Key to the species of *Entomophthora* sensu lato. Bull. Br. Mycol. Soc., 16: 113–143.

Wilding, N. 1973. The survival of *Entomophthora* spp. in mummified aphids at different temperatures and humidities. J. Invertebr. Pathol., 21: 309–311.

Received 25 June 1991; accepted 13 August 1991.